cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas

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cIMPACT-NOW Update 5:

Recommended Grading Criteria and Terminologies for

IDH-mutant Astrocytomas

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Introduction

The diagnostic importance of IDH mutational status in diffuse gliomas was first formally recognized within the updated 4th edition of the WHO Classification of Tumours of the Central Nervous System (2016). Its introduction as a diagnostic marker was based on evidence that incorporation of biomarkers into an integrated diagnosis provided a more reproducible and clinically meaningful classification of diffuse gliomas in adults [20-22]. For IDH-mutant diffuse astrocytic gliomas, the integrated diagnostic entities (and corresponding grades) of the 2016 WHO Classification included: Diffuse Astrocytoma, IDH-mutant (WHO grade II), Anaplastic Astrocytoma, IDH-mutant (WHO grade III) and Glioblastoma, IDH-mutant (WHO grade IV). In contrast to IDH-mutant tumors, IDH-wildtype diffuse astrocytic gliomas are now recognized as distinct clinical and genetic entities that usually have much more aggressive clinical behavior, particularly in adults [5, 13]. While these molecular classifications represented a major step forward, grading schemes for the new diagnostic classes were not modified in parallel. The current grading criteria for diffuse astrocytic gliomas were developed prior to the understanding of molecularly distinct entities, yet the 2016 WHO update applies these same grading criteria for both IDH-mutant and IDH-wildtype gliomas [11, 14].

These legacy grading systems based on morphologic features (mitotic activity, anaplastic nuclear features, microvascular proliferation and necrosis) are not optimal [24, 27]. In particular, multiple retrospective studies have concluded that histologic grading criteria may not stratify risk for patients with IDH-mutant astrocytomas in the WHO grade II and III categories [1, 7, 24, 27, 33]. Yet, other studies have demonstrated that traditional grading schemes are still capable of stratifying risk for these patients [8, 30, 32]. In an attempt to improve risk stratification, several studies have investigated potential morphologic, proliferative or molecular markers that correlate with aggressive clinical behavior and could be incorporated into a more clinically relevant grading scheme [1, 2, 6, 7, 26, 30-32].

We evaluated the literature to determine whether there is sufficient evidence to define molecular genetic or other criteria that could reliably stratify risk among patients with IDH-mutant diffuse astrocytic gliomas or could identify those tumors that would behave most aggressively, with a clinical course corresponding to WHO grade IV. Among the molecular alterations considered were: *CDKN2A/B* homozygous deletion, *CDK4* amplification, *RB1* mutation or homozyous deletion, *PIK3CA* or *PIK3R1* mutations, *PDGFRA* amplification, *MYCN* amplification, global DNA methylation levels, genomic instability and chromosome 14 loss. We also considered whether there were thresholds of proliferative activity, based on mitotic count or Ki-67 indices, or other morphologic features typical of a high grade that might stratify risk better than current criteria. Finally, we considered potential future nosologies for IDH-mutant diffuse astrocytic gliomas in order to more clearly delineate these from IDH-wildtype diffuse gliomas. To achieve these goals, cIMPACT-NOW assembled a group of experienced neuropathologists and clinical neuro-oncologists as Working Committee 1 for Round 2 discussions, which held three teleconferences in an open manner similar to the discussions held at WHO consensus meetings. A subsequent meeting of cIMPACT-NOW in Utrecht, the Netherlands in September 2019 was used to further shape the recommendations and justifications of Working Committee 1.

Molecular Alterations Discussed for Grading of IDH-Mutant Diffuse Astrocytomas

CDKN2A/B homozygous deletion

Multiple studies have identified homozygous deletion of *CDKN2A/B* as a marker of poor prognosis in patients with IDH-mutant diffuse astrocytic gliomas [1, 2, 8, 16, 26, 30, 32, 33]. Initial observations were that both *CDKN2A/B* homozygous deletions and *CDK4* amplification were enriched among IDH-mutant astrocytomas that were associated with poor prognosis, and that this subset also showed lower levels of global DNA methylation (G-CIMP-low) [6]. Subsequent investigations of *CDKN2A/B* homozygous deletion as an independent marker in WHO grade II and III IDH-mutant astrocytic gliomas confirmed a

strong association with shorter survival [7, 8, 26, 33]. A more recent study demonstrated that *CDKN2A/B* homozygous deletion was strongly associated with a poor prognosis in a cohort that included all grades of IDH-mutant astrocytomas (WHO grades II-IV) on univariable analysis [30]. In particular, *CDKN2A/B* homozygous deletions in histologic grade III IDH-mutant astrocytomas were associated with shorter patient survival, similar to WHO grade IV tumors [30]. Other investigations have corroborated these findings [2, 16, 30]. The frequencies of *CDKN2A/B* homozygous deletions reported in IDH-mutant astrocytic gliomas range from 0-12% in WHO grade II, 6-20% in WHO grade III and 16-34% in WHO grade IV tumors [2, 30, 32]. It should be noted that the prognostic associations reported for *CNKN2A/B* homozygous deletion have been based on retrospective cohorts with potentially confounding prognostic parameters, notably age and divergent patterns of care. Moreover, homozygous deletion at 9p21 not only targets the *CDKN2A/B* locus, but also other neighboring genes that have known or suspected tumor suppressive functions [3, 15, 29].

Alteration of other RB pathway genes

CDK4 amplification in IDH-mutant astrocytomas was associated with poor prognosis and its combination with chromosome 14 loss predicted an even shorter overall survival [7, 8]. Other studies have concluded that *CDK4* amplification was not associated with poor prognosis [2, 30]. Homozygous deletion of *RB1* was strongly associated with inferior overall survival among IDH-mutant astrocytomas on univariate analysis**,** but this finding was not corroborated in other investigations [2, 30]. In a multivariate analysis of two sizable patient cohorts, Aoki et al. demonstrated that altered RB pathway genes (*CDKN2A/B* homozygous deletion, *CDK4* amplification or *RB1* mutation), when considered together, were a strong and statistically significant predictor of poor prognosis in IDH-mutant astrocytoma patients [1]. When considered by themselves in this study, each of these markers was associated with a less favorable prognosis, although not significantly on univariate analysis. The prognostic role of less common RB pathway gene alterations, such as *CDKN2A/B* point mutation, *CDKN2A/B* promoter methylation or *CDK6* amplification remains unclear and deserves further study.

PIK3R1 and *PIK3CA* mutations

On multivariate analysis, *PIK3R1* mutations were an independent marker of poor prognosis in IDH-mutant astrocytomas of WHO grade II or III. *PIK3CA* mutations showed a strong trend towards shorter overall survival but were not an independent marker on multivariable analysis [1].

PDGFRA amplification

Multiple studies have demonstrated that *PDGFRA* amplification is associated with shorter survival among patients with IDH-mutant astrocytic gliomas, including a recent investigation showing its prognostic significance specifically in histologic grade II and III tumors on multivariable analysis [25, 30, 32]. Another study did not uncover this association [1].

MYCN amplification

MYCN amplification was associated with shorter overall survival in patients with IDH-mutant astrocytomas (WHO grades II-IV) on univariable analysis [30].

Genomic instability

Both high levels of copy number variations (CNV) and somatic mutations have been associated with higher histologic grade among IDH-mutant astrocytomas and with shorter overall survival in patients with WHO grade II or III IDH-mutant astrocytomas [1, 9, 28]. In a separate investigation, patients with IDH-mutant astrocytomas that displayed a high CNV level had shorter overall survival than those with low CNV level [30]. There are challenges in the comparison and interpretation of these investigations, since the thresholds for high CNV and somatic mutation varied [23].

Reduced global DNA methylation

In a study of 1,122 diffuse gliomas, a small subset of IDH-mutant diffuse astrocytic gliomas (WHO grades II-IV) were found to have globally reduced levels of DNA methylation (G-CIMP-low) relative to the

majority of IDH-mutant astrocytomas, as well as a distinctive gene expression profile [6]. Half of these G-CIMP-low gliomas corresponded to WHO grade IV and the other half were histologically WHO grade II or III. Patients with G-CIMP-low IDH-mutant astrocytomas had shorter overall survival than patients in the G-CIMP-high group. More than 75% of the G-CIMP-low tumors had alterations in RB pathway genes (*CDKN2A/B* homozygous deletion and *CDK4* amplification). Another study, focused exclusively on IDH-mutant glioblastoma, WHO grade IV, confirmed both the short survival of patients with G-CIMP-low tumors and the association with *CDKN2A/B* homozygous deletion [17].

Other genetic markers

Other genetic markers of interest did not show strong evidence for the ability to stratify risk among patients with IDH-mutant astrocytomas or predict WHO grade IV behavior. Larger or additional studies may provide stronger evidence in the future [6, 8, 12, 24, 30].

Mitotic activity and proliferation indices

The traditional method for stratifying risk among histologic grade II or III diffuse astrocytic gliomas has relied heavily on the identification of mitotic activity. The WHO 2016 indicates that "significant proliferative activity" distinguishes anaplastic astrocytoma, IDH-mutant, WHO grade III from diffuse astrocytoma, IDH mutant, WHO grade II [20]. Based on studies in the pre-WHO 2016 era, astrocytomas with ≥ 2 mitoses in the entire specimen have been shown to be associated with shorter survival than those with 0 or 1 mitoses and this threshold has therefore been used by practicing neuropathologists for the designation of WHO grade III [10, 11, 14]. Specimen size must also be considered. In a very small biopsy, one mitosis may be sufficient, whereas in very large specimens, greater mitotic activity may be necessary [20]. These thresholds for mitotic activity have not been corroborated by several studies of IDH-mutant cohorts [12, 24, 33]. However, others have demonstrated that traditional grading schemes can stratify risk among patients with grade II and III IDH-mutant astrocytomas, but with ample opportunity for improvement [8, 30, 32]. To date, there have been no studies that establish an alternative mitotic count that

more reliably stratifies risk among histologic grade II and III IDH-mutant astrocytomas. Similarly, studies of proliferative index (e.g. based on Ki-67) have not identified criteria that unequivocally stratify risk among patients with IDH-mutant astrocytomas [12].

Summary of findings

The currently available evidence from multiple retrospective studies suggests that homozygous deletion of *CDKN2A/B* is associated with shorter survival in patients with IDH-mutant astrocytomas and that its presence corresponds to WHO grade IV clinical behavior. Alterations in other genes encoding members of the RB pathway, including *CDK4* amplification or *RB1* mutation/homozygous deletion, may also be markers of aggressive clinical behavior but the evidence is not as firmly established (e.g., fewer cases or fewer published studies). Several studies have demonstrated *PDGFRA* amplification as a marker of poor prognosis with potential for inclusion as a grading criterion with additional corroborating evidence. While mutations in *PIK3R1* and *PIK3CA*, as well as amplifications in *MYCN*, have been associated with shorter survival, additional cohorts are needed for validation. Genomic instability is a feature corresponding to poor prognosis in patients with IDH-mutant astrocytomas. However, the analyses and thresholds for clinical validation of genomic instability have not been firmly established for application to clinical practice. Similarly, G-CIMP-low DNA methylation pattern has been associated with shorter survival in IDH-mutant astrocytoma, but additional cohorts are needed for validation to more precisely define the G-CIMP-low methylation diagnostic profile as well as to assess the practicality of testing modalities. There is currently insufficient evidence to establish a new threshold of mitotic activity to discriminate histologic grade II and III IDH-mutant astrocytomas. Overall, with regard to clinical outcomes and grading criteria, we have been cautious in our interpretation of the literature, since most large studies on the relationship between genetic alterations and clinical outcomes have relied on retrospective cohorts in which patients had been treated differently depending on institution, era and histologic classification. Moreover, clinical follow-up times are limited in most studies, which is a particular weakness when assessing prognostic markers in patients whose median overall survival is beyond 10 years.

Proposed Terminology for next WHO classification

The terms used to classify the diffusely infiltrative gliomas are deeply rooted in history and based on presumed tumor cell lineage and levels of differentiation. For the diffuse astrocytic gliomas, we now understand that IDH-wildtype and IDH-mutant tumors represent distinct clinical and genetic entities, despite the similar terms used for their classification by the WHO (diffuse astrocytoma, anaplastic astrocytoma and glioblastoma). Terminologies that more clearly distinguish IDH-mutant and IDH-wildtype diffuse astrocytic gliomas are desirable. One suggestion was to reserve the term "glioblastoma" for those diffuse astrocytic gliomas that are IDH-wildtype and have histologic or genetic features predictive of a highly aggressive clinical behavior corresponding to WHO grade IV [4]. Diffuse astrocytic gliomas that are IDH-mutant would be graded based upon morphologic and genetic features that corresponded to WHO grade II, III or IV clinical behavior. The suggested terminologies, class definitions, and grading criteria for IDH-mutant astrocytomas are summarized in Table 1. We recognize that changes of this type may be viewed as controversial and will require further discussion in context of the next WHO classification, which is scheduled for later 2020 (see Supplemental Text for critiques and responses). Note the use of the Arabic numerals 2, 3 and 4, rather than the Roman numerals II, III and IV, that had traditionally been used for WHO CNS tumor grades; Arabic numerals are suggested in order to harmonize with WHO grading schemes of other tumor types and to reduce the possibility of introducing typographical and interpretive errors (i.e., the distinction of *2* vs *3* is less susceptible to error in a report than *II* vs. *III*).

Table 1. IDH-mutant Astrocytomas

Astrocytoma, IDH-mutant, WHO grade 2

A diffusely infiltrative astrocytic glioma with an *IDH1* or *IDH2* mutation that is well differentiated and lacks histologic features of anaplasia. Mitotic activity is not detected or low*. Microvascular proliferation, necrosis and *CDKN2A/B* homozygous deletions are absent.

Astrocytoma, IDH-mutant, WHO grade 3

A diffusely infiltrative astrocytic glioma with an *IDH1* or *IDH2* mutation that exhibits focal or dispersed anaplasia and displays significant mitotic activity*. Microvascular proliferation, necrosis and *CDKN2A/B* homozygous deletions are absent.

Astrocytoma, IDH-mutant, WHO grade 4

A diffusely infiltrative astrocytic glioma with an *IDH1* or *IDH2* mutation that exhibits microvascular proliferation or necrosis or *CDKN2A/B* homozygous deletion or any combination of these features.

*= see text regarding mitotic count cut-off values

Grading considerations for IDH-mutant astrocytomas.

IDH-mutant astrocytomas that lack significant mitotic activity, histologic anaplasia, microvascular proliferation, necrosis and *CDKN2A/B* homozygous deletion are referred to as Astrocytoma, IDH-mutant, WHO grade 2. Patients with these tumors have a median overall survival greater than 10 years [2, 30]. An IDH-mutant astrocytoma that contains elevated mitotic activity and histologic anaplasia, yet lacks microvascular proliferation, necrosis and *CDKN2A/B* homozygous deletion, currently fits into the designation of Astrocytoma, IDH-mutant, WHO grade 3. Recognizing that no validated published criteria exist for mitotic count cut-off values for grading IDH-mutant astrocytomas, “significant” mitotic activity remains the criterion to distinguish WHO grade 3 from grade 2 tumors. Most neuropathologists use a

threshold of ≥ 2 mitoses within the entire specimen, or 1 mitosis in very small biopsies, while large specimen may require more [10, 14, 20]. The extent to which Astrocytoma, IDH-mutant, WHO grade 3 exhibits clinically more aggressive behavior relative to its WHO grade 2 counterpart remains to be determined. It should be noted that future studies may refine mitotic thresholds for grading and may identify additional genetic alterations associated with more aggressive clinical behavior among WHO grade 2 and 3 IDH-mutant astrocytomas.

IDH-mutant astrocytomas with microvascular proliferation or necrosis or *CDKN2A/B* homozygous deletion, or any combination of these features, correspond to WHO grade 4. These tumors have been formerly considered as "Glioblastoma, IDH-mutant, WHO grade IV". However, they are clinically and genetically distinct from glioblastoma, IDH-wildtype, and closely related to WHO grade 2 or 3 IDH-mutant astrocytomas. Thus, cIMPACT-NOW recommends that the WHO strongly consider discontinuing the term "Glioblastoma, IDH-mutant, WHO grade IV" and instead recommends referring to these tumors as “Astrocytoma, IDH-mutant, WHO grade 4”. Based on the strength of evidence, cIMPACT-NOW also recommends that *CDKN2A/B* homozygous deletion should be a WHO grade 4 criterion for IDH-mutant astrocytomas. Some studies have concluded that homozygous deletion of *CDKN2A/B* is associated with worse outcome even among patients with histologically defined WHO grade 4 IDH-mutant astrocytomas [16, 30]. Homozygous deletion can be determined by FISH, quantitative PCR, MLPA, microarray- or NGS-based methods. However, immunohistochemistry for p16 does not correlate well with deletion [26].

These recommendations represent the initial steps toward advancing our ability to distinguish clinically relevant subgroups of IDH-mutant astrocytomas at a diagnostic level, and in turn guide patient care and inclusion into clinical trials. In combination with the other cIMPACT-NOW updates, it is further anticipated that such recommendations will contribute to decisions guiding the 5th edition of the WHO brain tumor classification.

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Supplemental Text

Reviewers' Primary Critiques and Authors' Responses to Proposed Grading and Terminologies in cIMPACT-NOW Update 5

Critique: The WHO 2016 Update of the 4th Edition introduced IDH-wildtype and IDH-mutant astrocytic gliomas as distinct entities, which is sufficient for diagnostic and treatment purposes. There is no need to eliminate the term glioblastoma for IDH-mutant astrocytic gliomas, since this will result in confusion.

Response: In 2016, the Updated 4th Edition of the WHO introduced IDH-mutant and IDH-wildtype forms of diffuse astrocytoma, anaplastic astrocytoma and glioblastoma, which was a justified and necessary first step in delineating these diseases. However, cIMPACT-NOW Working Committee 1 was concerned that the exact same names were applied to diseases that were distinct. We concluded that referring to IDH-mutant and IDH-wildtype forms of these diseases by using the same terms (e.g. glioblastoma) perpetuates the misconception of these tumors as variants of the same disease process (i.e. "the good prognosis form of GBM" or "GBM with a prognostically favorable mutation"). Rather, IDH-mutant and IDH-wildtype forms of astrocytic gliomas differ based on their clinical demographics and presentation, genetics, epigenetics and behavior, and will require separate treatments and clinical trials based on their diagnosis. Given that IDH-mutant and IDH-wildtype astrocytic gliomas are separate and specific diseases, our Committee concluded that they should have separate and specific terminologies. We proposed to reserve the term Glioblastoma for IDH-wildtype astrocytic gliomas of grade IV. With regard to clinical care and enrollment on clinical trials, the Committee concluded that there would be less confusion by eliminating the use of similar terms for different diseases.

Critique: The rationale for using Arabic numerals instead of Roman numerals is not strong. This change will result in confusion, since pathologists and clinicians are familiar with Roman numerals for grading.

Response: The WHO has strongly signaled that standardization of terminologies and style will be expected across all tumor types going forward in the upcoming 5th Edition. The vast majority of WHO tumor classifications have used Arabic for grading purposes and this will almost certainly be the standardized practice moving forward. For example, the first two publications of the 5th Edition WHO Classification—Breast Tumours and Tumours of the Digestive System--have both used Arabic numerals for those tumors that require numerical grading. In addition, there is a practical point in using Arabic numerals in that typographical and interpretive errors are much less likely with the use of Arabic numerals (grades 2 vs 3) than with Roman numerals (grades II vs III).

Critique: cIMPACT-NOW should have been bolder and more ambitious in their recommendations for molecular grading criteria, since it is well known that morphologic grading criteria are not optimal for stratifying risk. Wouldn't it be easier to classify and grade these tumors on the basis of the molecular profiling only?

Response: cIMPACT-NOW Working Committee 1 evaluated the literature to determine if there were morphologic or genetic markers that could more optimally stratify risk among IDH-mutant astrocytomas and be utilized to improve grading criteria. We concluded that the evidence was strong for *CDKN2A/B* homozygous deletion as a marker of grade 4 behavior and have included it in the proposal for a revised grading scheme. We did not identify other morphologic, proliferative or genetic markers that have been reproducibly demonstrated to improve risk stratification in IDH-mutant astrocytomas and could be used clinically. Morphologic markers still play a large role in grading IDH-mutant astrocytomas. Microvascular proliferation and necrosis are associated with grade 4 behavior and have excellent reproducibility and predictive value. We agree that additional studies will be necessary to improve prognostication, risk stratification and grading schemes for grade 2 and grade 3 IDH-mutant astrocytomas.

Critique: The morphologic criteria for distinguishing grade 2 from grade 3 have not changed from the WHO 2016 4th Edition Update. Why doesn't cIMPACT provide new criteria based on mitoses per high power field (HPF) or mitoses per mm^2?

Response: A major conclusion from our cIMPACT-NOW discussions was that there was no solid evidence in the literature for improved grading criteria to distinguish grade 2 and grade 3 IDH-mutant astrocytomas. Therefore, the morphologic criteria for distinguishing grade 2 from grade 3 in cIMPACT-NOW Update 5 are the same as those in the 2016 WHO classification. With regard to providing a "mitotic count/HPF" or "mitotic count/mm^2", we are not aware of studies that have shown such thresholds for discriminating grade 2 from grade 3 IDH-mutant astrocytomas. Lacking evidence for change, we opted to continue with WHO 2016 criteria, with the exception of including *CDKN2A/B* homozygous deletion as a grade 4 criterion.

Critique: The criterion of "≥2 mitoses" for distinguishing grade 2 from grade 3 IDH-mutant astrocytomas is confusing. Is this ≥2 mitoses per field, per 10 high-power fields, or per entire specimen? Who established these criteria? This is not common practice. The references cited for these criteria are old and questionable.

Response: The criteria used for grading diffuse astrocytic neoplasms has evolved over time. The WHO 1st edition (1979) used "areas of anaplasia" as the criterion for establishing a diagnosis of anaplastic astrocytoma, grade 3. Mitotic activity was not a criterion in the 1st edition. In a study of morphologic features of astrocytic neoplasms and their associations with clinical outcomes, Daumas-Duport et al concluded that nuclear atypia and <u>any</u> mitotic activity (these were 2 of the four features included in the study, along with microvascular hyperplasia and necrosis) could be used to stratify grade 3 from grade 2 and led to the Mayo-St. Anne criteria (1988)[5]. Importantly, this study concluded that the presence of <u>any mitoses identified within the entire specimen</u> (≥ 1) was associated with a shorter survival and distinguished grade 3 from grade 2 clinical behavior among astrocytomas. Based on these findings, the WHO 2nd edition (1993) used "mitotic activity" (any in the specimen) to distinguish anaplastic astrocytoma, grade 3 from astrocytoma, grade 2. The findings of the Mayo-St. Anne criteria were confirmed in an independent cohort in 1998 by Coons and Pearl, who demonstrated that the presence of 1 mitosis in the entire specimen stratified patients with long survival (grade 2) from those with intermediate survival (grade 3)[4]. In 1998, Giannini et al investigated whether other levels of mitotic

activity might stratify risk better than any (≥ 1) mitoses per specimen [6]. They found that astrocytomas with ≤ 1 (either 0 or 1) mitoses in the entire specimen were associated with survivals similar to grade 2 astrocytomas and longer than anaplastic astrocytoma, grade 3. These results indicated that the finding of 1 mitotic figure was not sufficient to predict grade 3 behavior in astrocytomas. This led the WHO 3rd edition (2000) to add "a single mitosis does not yet allow the diagnosis of anaplastic astrocytoma" (i.e. grade 3) and also stated that a grade 2 designation was appropriate if mitoses are "very rare or absent". In this same edition, anaplastic astrocytoma, grade 3, was defined as having "marked mitotic activity", with no precise values provided. In the WHO 4th edition (2007), the criteria for diffuse astrocytoma, WHO grade 2 indicated that "a single mitosis does not allow the diagnosis of anaplastic astrocytoma", without an area provided and therefore continued the practice of using ≥ 2 mitoses in the entire specimen as the threshold. In the updated 4th edition of the WHO (2016), Diffuse astrocytoma, IDH mutant, WHO grade 2 and Anaplastic astrocytoma, IDH mutant, WHO grade 3 were introduced. Since there were no definitive studies of morphologic features (i.e. mitoses, etc) and clinical outcomes on IDH-mutant astrocytomas, the criteria for distinguishing grade 3 from grade 2 remained largely the same. For grade 2: "Mitotic activity is generally absent but a single mitosis does not justify the diagnosis of anaplastic astrocytoma unless observed in a small biopsy or in the setting of obvious nuclear anaplasia". In the description of anaplastic astrocytoma, IDH mutant, WHO grade 3, it is stated "The principal histopathological features are those of a diffusely infiltrating astrocytoma with increased mitotic activity compared with the WHO II equivalent, usually accompanied by distinct nuclear atypia and high cellularity. Mitotic activity should be evaluated in the context of sample size". It was here that the idea that sampling size should be taken into consideration for establishing a grade 2 or grade 3 using mitotic activity. These statements were justified based on the findings of Coons and Pearl (1998) that the identification of mitoses depended upon the number of fields viewed[4].

Thus, the mitotic threshold for distinguishing grade 2 and 3 astrocytomas is ≥ 2 mitoses in the entire specimen, with appropriate consideration to the size of specimen. We have not deviated from the WHO 2016 nor from the tradition that it has continued. There are a few studies that have shown that the WHO criteria can be used to stratify risk among patients with grade 2 versus 3 IDH-mutant astrocytomas [3, 9, 10]. In contrast, other studies have not been able to demonstrate a significant prognostic role of WHO grading and/or mitotic count in IDH-mutant astrocytoma patients [1, 2, 7, 8, 11]. Because there is not sufficient evidence to introduce a change in grading criteria for grade 2 and grade 3 IDH-mutant astrocytomas, we have adhered to the WHO 2016 criteria and acknowledge the need for additional studies. We agree that several of the references cited are old and may be questionable in the current molecular era. There are simply no better data currently available.

Critique: When proposing a change in grading, which is intended to correspond to clinical behavior (in this case grades 2, 3, and 4), a comparative survival analysis should be performed with other tumors to ensure consistency of outcomes with respect to grade. The current grading proposal (2, 3, 4) is not normalized to clinical outcome with other entities.

Response: Grading of neoplasms based upon WHO guidelines and criteria is not precisely uniform or normalized with regard to clinical outcomes across CNS (or non-CNS) tumors. Grading is intended to reflect the natural history of untreated neoplasms. We do not currently have good evidence on the natural history of IDH-mutant astrocytomas. We rely heavily on the clinical outcomes from retrospective

studies of treated patient cohorts to provide correlations of clinical outcomes with specific grading criteria. Grading criteria will be improved with the availability of solid evidence published and validated in the literature. There are many examples of tumors with similar grades that have differing clinical outcomes. For example, the genetic and morphologic subtypes of medulloblastoma are all considered grade 4 based on their natural histories, yet are associated with substantially different clinical outcomes when patients are being treated according to current standard of care. Even with the best data available, it would not be expected that a cohort of patients with IDH-mutant astrocytoma, WHO grade 4 would have clinical outcomes similar or identical to a cohort of patients with glioblastoma, IDH-wildtype, WHO grade 4. However, both cohorts have diseases that are associated with aggressive clinical courses and are treated based on the message conveyed by the tumor grade.

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